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Proportion of Cells With Paternal 11p15 Uniparental Disomy Correlates With Organ Enlargement in Wiedemann-Beckwith Syndrome

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“Genetic mosaicism” describes the presence of two or more populations of cells within a single individual that differ in their genomic constitution. Although the occurrence of asymmetric overgrowth in Wiedemann-Beckwith syndrome (WBS) suggests that mosaicism has some role in the WBS phenotype, no direct evidence for this has been published. WBS is a congenital overgrowth syndrome with variable phenotype linked to the imprinted gene cluster on chromosome region 11p15. We have performed a molecular survey of multiple organs and tissues in a case of WBS with a high degree of mosaic paternal 11p15 uniparental disomy (UPD). The organs most severely affected were those with the highest percentage of cells with UPD. In particular there was a striking difference in the degree of mosaicism for 11p15 UPD between the extremely enlarged left adrenal and non-enlarged right adrenal gland. This result indicates that the proportion of paternal 11p15 UPD cells correlates with the tissue phenotype of WBS. Our results suggest that high proportions of abnormal cells result from a combination of stochastic events and cell selection. Mosaicism may explain the variable phenotypes including hemihyperplasia and predisposition to childhood cancers in WBS patients. Am. J. Med. Genet. 92:111–116, 2000. © 2000 Wiley-Liss, Inc.

KEY WORDS: uniparental disomy; Wiedemann-Beckwith syndrome; mosaicism, 11p15; asymmetry; overgrowth; adrenal hyperplasia

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INTRODUCTION

Wiedemann-Beckwith syndrome (WBS) is a congenital overgrowth syndrome that comprises high birth weight, visceromegaly, macroglossia, abdominal wall defects, hemihyperplasia, cytomegaly of the fetal adrenal cortex, hyperplasia of pancreatic islets, and a predisposition to childhood tumors [Pettenati et al., 1986]. Genetic studies indicate linkage of WBS to 11p15 [Koufos et al., 1989; Ping et al., 1989], the location of an imprinted region that contains at least eight imprinted genes including the paternally expressed gene *IGF2* and the maternally expressed genes *H19* and *CDKN1C* (*p57^{KIP2}*) [Morison and Reeve, 1998a]. Because several different genetic mechanisms, including uniparental disomy (UPD) of chromosome 11p [Henry et al., 1991], relaxation of *IGF2*/*H19* imprinting [Weksberg et al., 1993], trisomy 11p [Okano et al., 1986], and *CDKN1C* mutation [Hatada et al., 1996; Zhang et al., 1997], are associated with WBS, it has been suggested that some of the phenotypic variability of WBS may be explained by the different patterns of gene involvement [Hastie, 1997]. However, the frequent occurrence of asymmetric overgrowth, including hemihyperplasia, must be explained by other factors such as variation in the expression and the location of the genetic abnormality in different tissues, that is, mosaicism. We and others have demonstrated “epigenetic mosaicism” for relaxation of imprinting of *H19*/*IGF2* in children with WBS and somatic overgrowth [Morison et al., 1996; Okamoto et al., 1997; Reik et al., 1995]. Similarly, WBS patients with paternal 11p15 UPD always show mosaicism [Catchpoole et al., 1997; Henry et al., 1993; Slatter et al., 1994]. In view of the wide range of possible explanations for phenotypic variability in WBS, we have examined the relationship between the degree of mosaic paternal 11p15 UPD and the pathological findings in many tissues from a patient and have demonstrated

that the extent of mosaicism is strongly associated with the pathological phenotype.

MATERIALS AND METHODS

Assay for Allelic Imbalance of Chromosome 11p15

More than 20 mm^2 of tissue was dissected from formalin-fixed paraffin-embedded tissue sections and suspended in a buffer containing 75 mM NaCl, 25 mM EDTA, and 0.5% Tween 20. Paraffin was removed from tissues by microwave treatment and centrifugation, and DNA was extracted by phenol/chloroform extraction. PCR was done in a 25 μL reaction mixture containing 50 mM KCl, 10 mM Tris-HCl pH 8.3, 2.0 mM $MgCl_2$, 0.2 mM each dNTP, 0.5 μM each primer, 1.25 U AmpliTaq Gold (Perkin-Elmer, Oak Brook, IL), and 0.3 μL α^{32}P-dCTP. The tyrosine hydroxylase (TH) polymorphic microsatellite marker was amplified using primers as described by Edwards et al. [1991]. The initial denaturation was at 95°C for 12 min followed by 40 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 2 min with a final elongation step at 72°C for 10 min. The labelled amplified DNA samples were separated on a 6% denaturing polyacrylamide DNA sequencing gel. The intensity of TH alleles was measured using a phosphoimager.

Calculation of Percentage of Cells With Paternal UPD

After normalisation of the allele bands intensity by using DNA from a normal individual, the proportion of cells with paternal UPD was calculated by using following equation [Chao et al., 1993]: Percent mosaicism $= (k-1)/(k+1) \times 100$, where k is the ratio of the intensity of the parental alleles (paternal/maternal ratio) of the test sample.

RESULTS

Patient History

A twin pregnancy was identified in utero at 24 weeks gestation. The twins had equal size but one had an omphalocoele and a normal female karyotype. The female infant and her normal male twin were delivered after induction of labor at 32 weeks of gestation because of severe maternal toxaemia of pregnancy. Her placenta was markedly enlarged (1,033 g) compared with her twin's (246 g). She was diagnosed immediately as having WBS because of her birth weight of 2,435 g (twin's weight 1,800 g), omphalocoele, macroglossia, earlobe creases, and left hemihyperplasia involving both limbs, the labia majora, and the tongue. The kidneys, pancreas, and left adrenal were large. Intractable hypoglycemia continued throughout her life. Following surgical repair of the omphalocoele, sequential problems, bowel perforation, infection, chronic cholestasis, and major metabolic disturbance resulted in death at age 3 months when she weighed 5,255 g. Supplemental cortisone was given for most of the child's life.

Genotype–Phenotype Correlations.

In order to examine whether the patient's pathological phenotype was determined by the degree of mosaicism of a duplicated paternal 11p15, we measured the relative ratio of the intensity of alleles of the tyrosine hydroxylase gene polymorphism [Edwards et al., 1991] in the placenta and many tissues obtained at necropsy and thereby calculated the percentage of cells with paternal 11p15 UPD. DNA extracted from all tissues showed significant levels of mosaicism for paternal UPD (Figs. 1, 2). Strikingly the pathology of several abdominal organs paralleled the extent of mosaicism for cells with UPD. In particular, the left adrenal gland, which contained 88% (95% confidence interval: 87–89%) isodisomic cells, was markedly enlarged, weighed 12.5 g (normal 1–4 g [Stoner et al., 1953]), and had a cerebriform appearance due to severe cortical nodular hyperplasia of the adult cortex [Lack, 1997] (Fig. 3a). In contrast, the right adrenal which contained 30% (CI: 11–42%) UPD cells had normal size (2.2 g) and had a histologically normal adult cortex (Fig. 3b). Both adrenals showed cytomegaly of the fetal zone, typical of WBS [Beckwith, 1969]. The pancreas had 82% (CI: 80–84%) UPD cells and was very large (34 g) with extreme hyperplasia of the islets of Langerhans [Beckwith, 1969] (Fig. 4). Both kidneys were large and showed renal medullary cystic dysplasia typical of WBS [Beckwith, 1969] (right kidney 55 g, 71% (CI: 70–73%) UPD cells; left 42 g, 69% (CI: 64–75%) UPD cells). The 1,033 g placenta contained 47% (CI: 44–49%) UPD cells compared with a result of < 5% UPD cells in the 246 g structurally normal placenta of the twin (theoretically the non-identical twin should have 0% UPD cells). The large placenta showed stromal hyperplasia, cystic hydrops, and vascular abnormalities, which have been described in some cases of WBS [Jauniaux et al., 1997; McCowan and Becroft, 1994]. The lungs were small [left 41 g, 62% (CI: 41–71%) UPD, right 49 g, 38% (29–45%) UPD cells] and the liver large (324 g, 41% (30–50%) UPD cells] but both showed acquired abnormalities consistent with the severe postnatal illness.

DISCUSSION

The Phenotype Correlates With the Proportion of Isodisomic Cells

By surveying multiple organs in a case of WBS and by focussing on the discordant pathology of a pair of

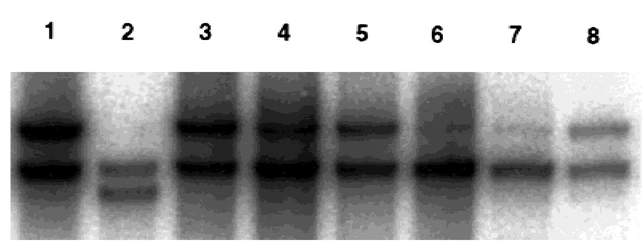

Fig. 1. Analysis of tyrosine hydroxylase polymorphic microsatellite marker in representative tissues. Lane 1, placenta of the unaffected twin; lanes 2, 3, and 4, blood from the father, mother, and patient; lanes 5, 6, 7, and 8, placenta, pancreas, and left and right adrenal cortices from the patient. The patient's upper and lower alleles were derived from her mother and father, respectively.

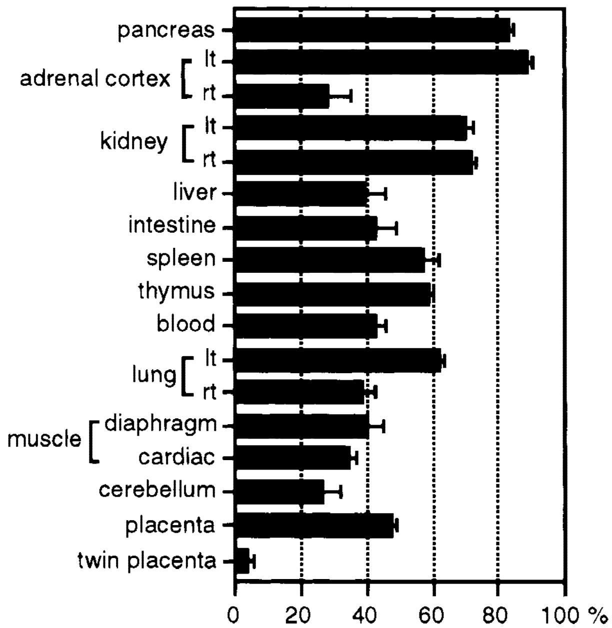

Fig. 2. Percent mosaicism of paternal 11p15 UPD cells in the patient's tissues. The percentage was calculated as shown in Materials and Methods. All data are expressed as means of three or more independent experiments + SEM.

adrenal glands, we have strong evidence that a high proportion of the genetically abnormal cells correlates with hyperplastic organ growth. Although it has been assumed that the asymmetry and phenotypic variability of WBS is attributable to mosaicism [Henry et al., 1993; Morison and Reeve, 1998b], we are not aware of previously published evidence that demonstrates this. For other conditions including Ullrich-Turner syndrome, trisomy 16, neurofibromatosis, type 1, and osteogenesis imperfecta [Benn, 1998; Colman et al., 1996; Edwards et al., 1992; Sarkar and Marimuthu, 1983; Wallis et al., 1990], there is evidence that mosaicism for a disease-causing abnormality or mutation might modify the disease, resulting in a less marked phenotype, but tissue specific correlations between genotype and phenotype have not been reported.

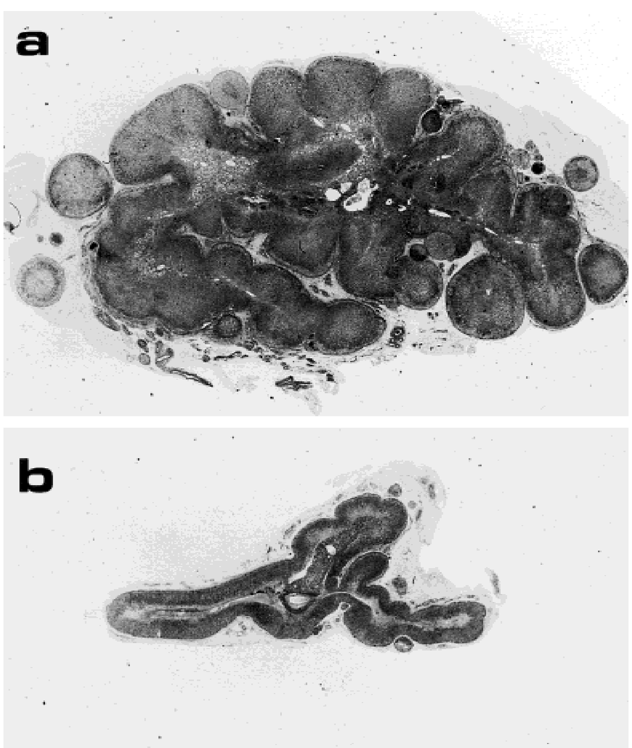

Fig. 3. Haematoxylin and eosin stained sections of the left adrenal gland showing the extremely convoluted and nodular structure of the definitive cortex, which gives a "cerebriform" appearance (**A**), compared with the normal contour of the right adrenal cortex (**B**). ×3.8.

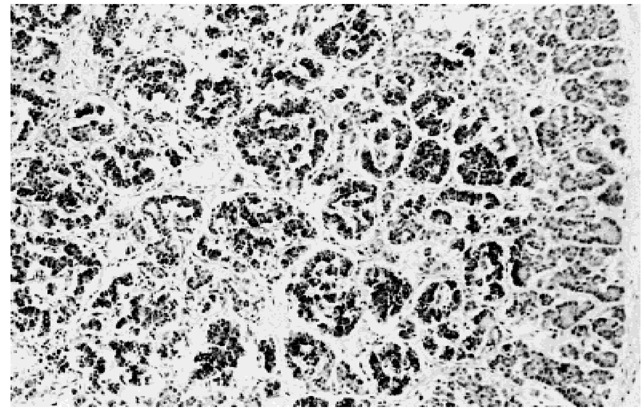

Fig. 4. Histological section of the pancreas stained for insulin (immunoperoxidase) showing that hyperplastic darkly-staining beta-cells outnumber the pale staining exocrine gland component. ×94.

Molecular Basis of Overgrowth in WBS

The UPD-associated overgrowth in WBS may be attributable to high levels of the imprinted, paternally expressed growth factor, insulin like growth factor 2 (IGF2). This assumption is supported by the observation that the only known trait common to all but one (*CDKN1C* mutation) of the molecular mechanisms associated with WBS is the potential for overexpression of IGF2 [Morison and Reeve, 1998b]. Furthermore, a clear relationship between IGF2 "dose" and WBS-like growth phenotypes was demonstrated in several mouse models [Eggenschwiler et al., 1997; Sun et al., 1997]. In addition to the effects of IGF2 overexpression, it is possible that a decreased levels of the maternally expressed gene *CDKN1C* ($p57^{KIP2}$) may contribute to the WBS phenotype [Hatada et al., 1996; Lee et al., 1997; O'Keefe et al., 1997; Zhang et al., 1997]. The developmental period during which overexpression of IGF2 contributes to overgrowth is not clear. Although IGF2 and its mitogenic receptor IGF1R are expressed as early as the pre-implementation embryo [Lighten et al., 1997; Rappolee et al., 1992], it is interesting to note that in a previously reported case of WBS, body overgrowth did not occur until after 22 weeks gestation [Hedborg et al., 1994], while in the case that we report here, the growth differential between the twins developed after 24 weeks gestation.

Origin of UPD Mosaicism

In the present case, mosaicism was present in both the placenta and the embryo and it is clear that the

somatic event leading to mosaicism must have occurred before cell commitment to the trophoectoderm and the inner cell mass. In mice commitment occurs in the 8–16 cell blastocyst and in humans the timing of blastocyst development is very similar [Hardy et al., 1989]. Thus the somatic recombination that causes UPD must have occurred during the first three or four postzygotic cell divisions [Kalousek, 1994]. In WBS, mosaic UPD typically involves only part of chromosome 11p and results from recombination between the heterochromatids during a somatic cell division [Bischoff et al., 1995]. The resulting daughter cells would each have paternal or maternal disomy for 11p, but available evidence suggests that cells with maternal UPD do not survive [Bischoff et al., 1995]. Somatic recombination that occurs during the first somatic cell division would result in 100% paternal UPD cells, a situation that has not been reported, whereas that which occurs in the second cell division would give rise to 33% of paternal UPD cells. Similarly an error during the third cell division would result in 14% UPD cells. To achieve the proportion of abnormal cells seen in this patient (at least 30% in most tissues), the abnormal disjunction could have occurred during the second cell division, unless there was a subsequent proliferative advantage for the abnormal cells.

Factors Influencing the Distribution of Mosaic Cell Populations

The levels of mosaicism varied widely between organs and tissues in this case but little is known about the factors that might determine this [Bernards and Gusella, 1994]. The first possibility is stochastic (chance) variation in the proportion of abnormal cells that contribute to the primordia of each tissue. A mouse model of mosaicism, in which post-fertilisation retroviral integration was used as a cell marker to study the distribution of mosaicism among multiple different organs in 16 mice, found two mice with significant variation in the amount of mosaicism [Soriano and Jaenisch, 1986]. This finding suggested that marked tissue variation due to stochastic processes can occur, but probably only in a minority of mosaic individuals.

The fact that most tissues in the body and placenta of our case had levels of mosaicism above the theoretical maximum of 33% provides strong evidence in favor of a second possibility: proliferative selection of cells with paternal UPD can contribute to high levels of mosaicism. In organs in which cells have a proliferative response to IGF2, the autocrine effects of IGF2 will lead to increased growth of mutant cells with UPD. The high levels of IGF2 in fetal kidney, adrenals, and pancreas suggest that IGF2 is especially important for the development of these organs [Hedborg et al., 1994], and thus selection may be more likely to have occurred in these organs.

Is There Evidence for a Threshold Effect?

In mosaic conditions, there is limited evidence to suggest that threshold mechanisms may lead to an "all-or-none" phenotype. For example, in mice with mosaic mutations of the Loop-tail gene, the occurrence of craniorachischisis was usually associated with proportions of mutant cells greater than a threshold of 50–60% [Musci and Mullen, 1990]. Determination of phenotypic thresholds may be especially important for those few conditions in which mosaicism is a characteristic feature. Examples include Wiedemann-Beckwith syndrome, McCune-Albright syndrome in which affected individuals are always mosaic for *GNAS1* mutations [Happle, 1986], and "Epidermal nevus of the epidermolytic hyperkeratotic type" in which affected individuals are always mosaic for keratin 10 mutations [Paller et al., 1994].

Can reliable correlations be established between the proportion of mosaic cells and the phenotype in an organ or an individual? At this time, there are few data from WBS cases from which organ-specific thresholds for UPD can be determined, and there is the difficulty that the measured levels of mosaicism may be altered by selection and might not reflect the levels present during critical developmental periods. Several tissues such as the lungs, thymus, blood, and brain are usually morphologically normal in cases of WBS [Pettenati et al., 1986], suggesting that the development of these tissues is unaffected by the gene expression changes of WBS, regardless of the proportion of abnormal cells. Data from our case suggest that 30% mosaicism may be below the threshold for hyperplasia of the adult adrenal cortex, while 88%, as seen in the left adrenal, is above the threshold. In contrast both fetal adrenal cortices showed cytomegaly, one of the commonest pathological findings in WBS [Beckwith, 1969; Pettenati et al., 1986], perhaps consistent with a very low tissue threshold, although it is possible that cytomegaly of fetal cortical cells reflects systemic endocrine aberrations. The threshold level for the kidneys is not clear given that the abnormal kidneys in our cases had 70% cells with UPD, whereas Chao et al reported 41, 54, 67, and 77% "isoallelic" cells in kidneys described as normal from four children with Wilms tumors [Chao et al., 1993], possibly because the children were older and pathological changes had diminished with age.

Predicting Genotype–Phenotype Correlations

With respect to mosaic conditions, it has been suggested "that the study of multiple tissues is a necessary approach, the eventual goal being the appreciation of the relationship between the characteristics of a somatic mosaicism and the phenotype it imparts" [Greally et al., 1996]. If much of the variable phenotypes of WBS is primarily attributable to the stochastic origins of mosaicism then, despite the difficulties of interpretation, study of additional tissues from other affected patients may clarify the nature and consistency of genotype–phenotype correlations. Those patients who have *CDKN1C* mutations, a condition that is not mosaic, would be expected to exhibit a less variable phenotype than those with UPD or loss-of-imprinting, and more detailed descriptions of appropriately characterized patient groups is needed.

The most commonly recognized asymmetrical phenotype in WBS is hemihyperplasia. Hemihyperplasia has

been observed in association with 11p15 UPD [Henry et al., 1993] and also in children with constitutional relaxation of *IGF2* imprinting [Weksberg et al., 1993]. The tissues we obtained for this study did not have direct bearing on this question but in view of our demonstration of a genotype–phenotype correlation in the asymmetrical adrenals, it is reasonable to assume that hemihyperplasia is also attributable to the asymmetrical distribution of abnormal cells. To our knowledge this has not been formally demonstrated, although Hedborg and colleagues found greater IGF2 expression in the larger half of an asymmetric tongue [Hedborg et al., 1994]. The most important manifestation of WBS is the occurrence of childhood malignancy, and it would be useful if the proportion of mosaic cells in susceptible organs such as the kidney could be used to predict the risk of malignancy.

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